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Aridification driven diversification of fan-throated lizards from the Indian subcontinent

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ABSTRACT

The establishment of monsoon climate and the consequent aridification has been one of the most important climate change episodes in the Indian subcontinent. However, little is known about how these events might have shaped the diversification patterns among the widely distributed taxa. Fan-throated lizards (FTL) (Genus: *Sitana*, *Sarada*) are widespread, diurnal and restricted to the semi-arid zones of the Indian subcontinent. We sampled FTL in 107 localities across its range. We used molecular species delimitation method and delineated 15 species including six putative species. Thirteen of them were distinguishable based on morphology but two sister species were indistinguishable and have minor overlaps in distribution. Five fossils were used to calibrate and date the phylogeny. Diversification of fan-throated lizards lineage started ~18 mya and higher lineage diversification was observed after 11 my. The initial diversification corresponds to the time when monsoon climate was established and the latter was a period of intensification of monsoon and initiation of aridification. Thirteen out of the fifteen FTL species delimited are from Peninsular India; this is probably due to the landscape heterogeneity in this region. The species poor sister genus *Otocryptis* is paraphyletic and probably represents relict lineages which are now confined to forested areas. Thus, the seasonality led changes in habitat, from forests to open habitats appear to have driven diversification of fan-throated lizards.

1. Introduction

The Asian climate has been largely shaped by the uplift of the Himalayas, the rise of Tibetan plateau and glaciation in the Northern hemisphere (Zhisheng et al., 2001; Ali and Aitchison, 2008; Patnaik et al., 2012; Harrison et al., 1992). The establishment of monsoon climate was perhaps one of the most important outcomes of these events. The earliest evidence for the monsoon season in South Asia is during early Eocene (~55–52 mya) (Shukla et al., 2014) around the time the Indian plate collided with the Asian plate. Other studies suggest that monsoon-like pattern occurred much later around ~40 mya (Licht et al., 2014; Liu et al., 2017). While some authors construe that the origin of South Asian monsoon season is due to the uplift of Tibetan plateau (Kutzbach et al., 1993), others argue that it is also due to the changes in the land-sea distribution (Ramstein et al., 1997). The most recent hypothesis is that the establishment of South Asian monsoon is not related to the uplift of the Tibetan plateau but rather it is due to movement of the Indian plate into the tropical Northern Hemisphere from the tropical Southern Hemisphere (Liu et al., 2017). When the monsoon formed in South Asia (after Eocene) it was followed by pronounced seasonality. First there was a cooling and drying phase around the Eocene-Oligocene boundary (~35 mya: Licht et al., 2014). This was followed by increased seasonality linked with the uplift of the Himalayas and Tibetan plateau (~23 mya: Clift et al., 2008) and an accelerated aridification from late Miocene onwards (~10 mya: Nelson, 2007; Molnar et al., 1993; Dettman et al., 2001). The Indian and Asian monsoons strengthened around 9–8 mya (Zhisheng et al., 2001) and there was drastic drop in ocean temperatures around 5 mya (Zachos et al., 2001). The global climate change during the Miocene and the aridification which followed may have influenced the diversification of several biotas of the Indian subcontinent. Past studies have highlighted the expansion of C4 grasses in many parts of the world including Asia during late Miocene 8–6 mya (Quade et al., 1989; Cerling et al., 1997; Retallack, 2001; Edwards et al., 2010; Stromberg, 2011; Agarwal and Ramakrishnan, 2017). The first grazing mammals started appearing in the subcontinent around 10 mya and there is evidence of long-term climatic influence on the vegetation structure and mammalian ecological diversity in this region (Barry et al., 2002; Badgley et al., 2008). Patnaik (2003) compared extant and fossil murids from Siwaliks, India and reported that abundance and distribution of murids from the

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Table 1
Primers used for DNA amplification and sequencing.

Primers	Sequence (5′-3)	Size (bp)	Reference
ND2			
L4437b	AAGCAGTTGGGCCCATACC	~1026	Macey et al. (1997 and 2000)
H5540	TTTAGGGCTTTGAAGGC		
RAG1			
RAG13F	TCTGAATGGAAATTCAAGCTGTT	~1005	Groth and Barrowclough (1999)
RAG18R	GATGCTGCCTCGGTCGGCCACCTTT		
R35			
R35 F	GACTGTGGAYGAYCTGATCAGTGTGGTGCC	~665	Leaché (2009)
R35 R	GCCAAAATGAGSGAGAARCGCTTCTGAGC		
PDC			
PHO F2	AGATGAGCATGCAGGAGTATGA	~424	Bauer et al. (2007)
PHO R1	TCCACATCCACAGCAAAAAACTCCT		

Pliocene tend to overlap with the present. Patnaik (2003) suggests this is an indication of the establishment of monsoon climate similar to present during the early Pliocene. Furthermore, he also reports the appearance of hypsodonty and grazing components in murid molars in the late Miocene when there was a shift from C3-C4 plants (Patnaik, 2003).

The role of monsoon climate in shaping the current diversity and distributions of Indian biota remains unclear. Miocene aridification has shown to have influenced diversification in three groups of lizards (*Cyrtopodion*, *Cyrtodactylus* & *Ophisops*) in the Indian subcontinent (Agarwal et al., 2014; Agarwal and Karanth, 2015; Agarwal and Ramakrishnan, 2017). However, information is scarce on the groups that started diversifying more recently during the late Miocene and Pliocene.

In this regard, the fan-throated lizards (*Sitana* Cuvier, 1827) a widespread genus of small, terrestrial lizards is of much interest (Smith, 1935; Deepak et al., 2016a). They are inhabitants of open areas in the arid and semi-arid regions of India (Deepak et al., 2016a). They are distributed over much of India and in the plains and coasts of Sri Lanka. Until recently fan-throated lizards were thought to be a monotypic genus (Das, 1997). In the turn of this century three species were described from Nepal (Anders and Kästle, 2002; Schleich and Kästle, 1998; Schleich et al., 1998). In the past two years, six new species were described from India and Sri Lanka and the species diversity in *Sitana* increased from four to ten (Amarasinghe et al., 2015; Deepak et al., 2016a, 2016b). Additionally, a new genus of fan-throated lizard, *Sarada* Deepak, Giri and Karanth, 2016, with three species (Deepak et al., 2016a), was recently described. Thus, currently, the *Sarada-Sitana* complex consists of 13 species. The genus *Otocryptis*, a wet zone limited taxa distributed in Southwest India and Sri Lanka, has been shown to be the immediate sister of *Sarada-Sitana* complex (Macey et al., 2000; Grismer et al., 2016). Recent studies have shown the split between *Sitana* and *Otocryptis* is ~28 mya and this lineage originated from the wet zones in SE Asia (Grismer et al., 2016).

Given that the fan-throated lizards are widespread, semi-arid zone adapted and have multiple species distributed in a heterogeneous landscape with complex geological and climatic history, they are an ideal group for investigating speciation and biogeographic patterns, particularly with respect to the recently established arid zone. In the current study, we sampled fan-throated lizards from across their range covering different landscapes. We used multilocus genetic data to understand its phylogenetic relationship and diversification patterns. Additionally, species-delimitation tools based on gene trees and species trees were also implemented to characterize cryptic diversity in this group. We also compared morphological data to check if the delimited species are morphologically different. We wanted to address three main questions in this study 1. When did *Sarada* + *Sitana* start diversifying? And do their diversification dates correspond to the time when arid and semi-arid zones were getting established? 2. How many putative species of *Sitana* and *Sarada* are found in India? 3. What are the potential biogeographic barriers governing the current distribution of different *Sarada* + *Sitana* species?

2. Materials and methods

2.1. Taxon sampling, DNA sequencing, and sequence alignment

We generated 257 sequences from 109 individuals collected in 107 localities across the distributional range of *Sitana* and *Sarada* (Supplementary material, Table S1; Fig. S1). Type localities and other likely habitats were targeted and lizards were hand collected during various field trips between 2011 and 2016. In areas under the jurisdiction of forest department collections were made with permits from respective state forest departments. Genomic DNA was extracted from liver and tail tip tissue samples that were stored in 99.9% ethanol and refrigerated at −20 °C. DNeasy (Qiagen™) blood and tissue kit were used to extract DNA. The entire gene sequences for NADH dehydrogenase subunit 2 (ND2: ~1026 bp) mitochondrial gene and partial sequences of three nuclear genes: Recombination activating gene 1 (RAG1: ~1026), RNA fingerprint protein 35 (R35: 655 bp), and Phosducin (PDC: ~424 bp) were amplified by polymerase chain reaction (PCR) and sequenced. Primer sequences for these loci are listed in Table 1. The PCR cycles were same as in previous studies (Macey et al., 1997; Macey et al., 2000; Groth and Barrowclough, 1999; Bauer et al., 2007; Leaché, 2009).

2.2. Phylogenetic analysis

We reconstructed the molecular phylogeny of fan-throated lizards using the concatenated dataset with maximum likelihood (ML) and Bayesian methods (BI). *Otocryptis* was used as outgroup based on the findings by Macey et al. (2000). ND2 sequences for *Otocryptis wiegmanni* and other published sequences of *Sitana* were downloaded from GenBank (Supplementary material, Table S1). We were not able to get samples of three species of *Sitana* (*S. schleichi* Anders and Kästle, 2002, *S. fusca* Schleich and Kästle, 1998 and *S. devakai*, Amarsinghe, Ineich and Karunarathna, 2015) and one *Otocryptis* (*O. nigristigma* Bahir and Silva, 2005). Sequences were aligned using ClustalW and uncorrected genetic distances were calculated using MEGA 5 (Tamura et al., 2011). The program PartitionFinder v1.1.1 (Lanfear et al., 2012) was used to find the best partition scheme and model of sequence evolution for each partition. The optimal partitioning scheme included seven partitions (Supplementary material, Table S2). Likelihood analysis was undertaken in the program RAxML 1.3.1 (Stamatakis et al., 2005). This program employs only one model of sequence evolution, therefore we used GTR + G for all seven partitions. RAxML GUI (Silvestro and Michalak, 2012) was used to conduct a maximum likelihood analysis. We used the ML+ rapid bootstrap method to search for best scoring

maximum likelihood tree and assessed branch support using 1000 non-parametric bootstrap replicates.

A Bayesian tree was also generated using the program MrBayes 3.2 (Ronquist et al., 2012). The seven partitions were assigned to different models (Supplementary material, Table S2). For this analysis, two Markov chains were initiated from random starting trees and allowed to run for 10,00,000 generations sampling every 100 generations. The analysis was terminated when the standard deviation of split frequencies was less than 0.005, and the first 25% of the trees were discarded as "burn-in".

2.3. Species delimitation

Recent studies indicate that the diversity within the fan-throated lizards has been grossly underestimated (Deepak et al., 2016a, 2016b). Therefore, in order to better characterize the cryptic diversity in fan-throated lizards, we used species delimitation tools on the molecular data. We implemented two species delimitation methods to delineate species in the fan-throated lizard complex using the mitochondrial dataset. First, a Bayesian implementation of the Poisson Tree Process (bPTP) described by Zhang et al. (2013) was used to delimit species. An ML tree based on the ND2 mitochondrial gene built using 102 sequences (Supplementary material, Table S1) was used for bPTP on the web-server (http://species.h-its.org/ptp/) and the analysis was run for 50,000 MCMC generations with default thinning and burn-in. Second, a generalized mixed Yule coalescent (GMYC) model developed by Pons et al. (2006) to delineate genetic clusters was employed. The R package 'splits' was used to run the GMYC analysis with the single-threshold method (R Core Team, 2013; Ezard et al., 2009). The GMYC analysis utilizes an ultrametric tree that was built in BEAST 1.8 (Drummond and Rambaut, 2007; Drummond et al, 2012) with 10,00,000 generations and default tree priors. Identical haplotypes were removed for this analysis (Supplementary material, Table S1). Additionally, the three nuclear genes (RAG1, R35, and PDC) were used to build a species tree in *BEAST. Input files for species tree analyses in *BEAST were prepared using BEAUtiv1.8.2 (Drummond et al., 2012). We used a UPGMA starting gene tree, Yule species tree priors and piecewise linear and constant root as the population size model. The species population mean was specified with a gamma prior with shape = 2.0 and scale = 0.0020 (see Aydin et al., 2014). A lognormal prior (mean = 0.0, stdev = 1.0) was set for species yule birth rate. Other priors were left as default in the BEAUti settings. Five separate runs of *BEAST were undertaken based on five classification schemes each with a different number of species. The classification schemes included putative species based on the guide tree from GMYC and bPTP methods. Furthermore, three additional classifications were derived by either merging or splitting species retrieved in the bPTP analysis. These additional classifications were based on our knowledge of species distributions and external morphology. In order to identify other axis of species boundary, we used nine morphological characters and one criterion to compare different genera and species: 1. Dewlap coloration (single blue line or blue line with orange patches or blue, black & orange patches), 2. Dewlap size (tip of lower jaw till the end of dewlap), 3. Dewlap type (highly serrated vs less serrated margins), 4. Spines on the back of the head (large vs small/ reduced), 5. Number of body scales (vertebral scales and ventral scales), 6. Presence/absence of enlarged scales on the thigh and lateral body, 7. Hindlimb length, 8. Hindfoot length and 9. Geographic separation (sister species separated by a geographical barrier or by another species). Dewlap characters were for male-male comparisons, body scale counts were compared for males and females separately, and we used it in our species diagnosis even if it is useful to separate apart one sex. In total, we used 465 (366 males and 99 females) specimens in the comparative morphological study. Most of our sampling was carried out during the breeding season and minimum of two and maximum of 20 individuals per species were photographed to document dewlap color pattern.

We estimated Marginal Likelihood estimate (MLE) scores for each of the five classifications (models), using path sampling and stepping-stone sampling methods as described by Baele et al. (2012, 2013). Marginal Likelihood estimates scores were estimated from 100 path steps each run for 5,00,000 generations. The best classification scheme/model was picked using Bayes factor calculated for all pairwise comparisons of MLEs (Kass and Raftery, 1995).

2.4. Divergence dating and lineage diversification pattern

We constructed individual gene trees using RAxML and MrBayes. Apart from 15 fan-throated lizards, 67 other Iguanian lizard species were also utilized (Supplementary material, Table S3). This dataset consisted of three genes (ND2, RAG1 & R35) from a total of 82 iguanians. The dataset was partitioned into six partitions based on the results of our PartitionFinder analysis (Supplementary material, Table S4). The program BEASTv.1.8.2 was used for the dating analysis. The program was run using Yule speciation process and relaxed uncorrelated lognormal clock model. Fossil calibrations used in this analysis are as follows: (1) 99 mya (Stem of Agamidae; (Daza et al., 2016)), (2) 72 mya (Stem to *Leiolepis* and *Uromastyx*; (Gilmore,1943; Moody, 1980; Gao and Norell, 2000; Simões et al., 2015), (3) 37 mya (Stem of *Uromastyx*; (Head et al., 2013)), (4) 48 mya (Earliest stem for *Uromastyx*; (Averianov and Danilov, 1996)) and (5) 21 mya (Stem of *Intellagama lesueurii*; (Covacevich et al., 1990)). The prior distribution for all fossil calibration was set to an exponential with a mean value of 2. All calibration settings used in the BEAST analysis are provided in Supplementary material, Table S5.

The higher level relationships in our phylogeny agreed with that of Grismer et al. (2016) except with respect to the phylogenetic position of *Salea* and *Calotes*. In Grismer et al. (2016) *Salea* and *Calotes* are sisters whereas in our tree they are not retrieved as a monophyletic group. Given that Grismer et al. (2016) used a much larger dataset (genomic data) we give greater credence to their result and accordingly in our analysis, we constrained *Salea* + *Calotes* to be monophyletic. Two independent analyses were run for 15,00,00,000 generations sampling every 5000 trees, the effective sample size (ESS) values were evaluated using Tracer 1.6 (Rambaut et al., 2014). The tree files were combined using LogCombiner 1.8.2 and constructed a maximum clade credibility tree using TreeAnnotator v1.8.2. We used the package 'ape' (Paradis et al., 2004) in R 3.1.2 (R Core Team, 2013) to create lineage through time (LTT) plots for the delimited species of *Sitana* and *Sarada* and *Otocryptis*. We then used gamma statistics to identify if the rate of diversification in *Sitana* + *Sarada* and *Otocryptis* is constant or variable through time (Pybus and Harvey, 2000). We did not subject our data for further diversification analysis to check rate shifts because of fewer tips (small sample size). Package BAMM, the widely used method for diversification analysis, is designed to handle large datasets (Moore et al., 2016; Rabosky et al., 2017).

3. Results

3.1. Phylogenetic analysis and body size

Bayesian and ML analyses recovered five major clades with similar topologies, however, the relationship of *Sitana marudhamneydhal* within *Sitana ponticeriana* clade changed in this two analyses (Figs. 1 and S2). Overall, there was high support for the monophyly of the genera *Sitana* and *Sarada* in both BI and ML trees (Figs. 1 and S2). These genera were sister to each other. While species in the genus *Sitana* are widely distributed across the Indian subcontinent, the sister genus *Sarada* is restricted to parts of peninsular India (Fig. 2). In all the four gene trees (ND2, RAG1, R35, PDC) *Sitana* and *Sarada* were reciprocally monophyletic in both ML and BI analyses.

All species in the genus *Sarada* are large-bodied species, the average snout-vent length (SVL) of the smaller species (*Sarada darwini*) was

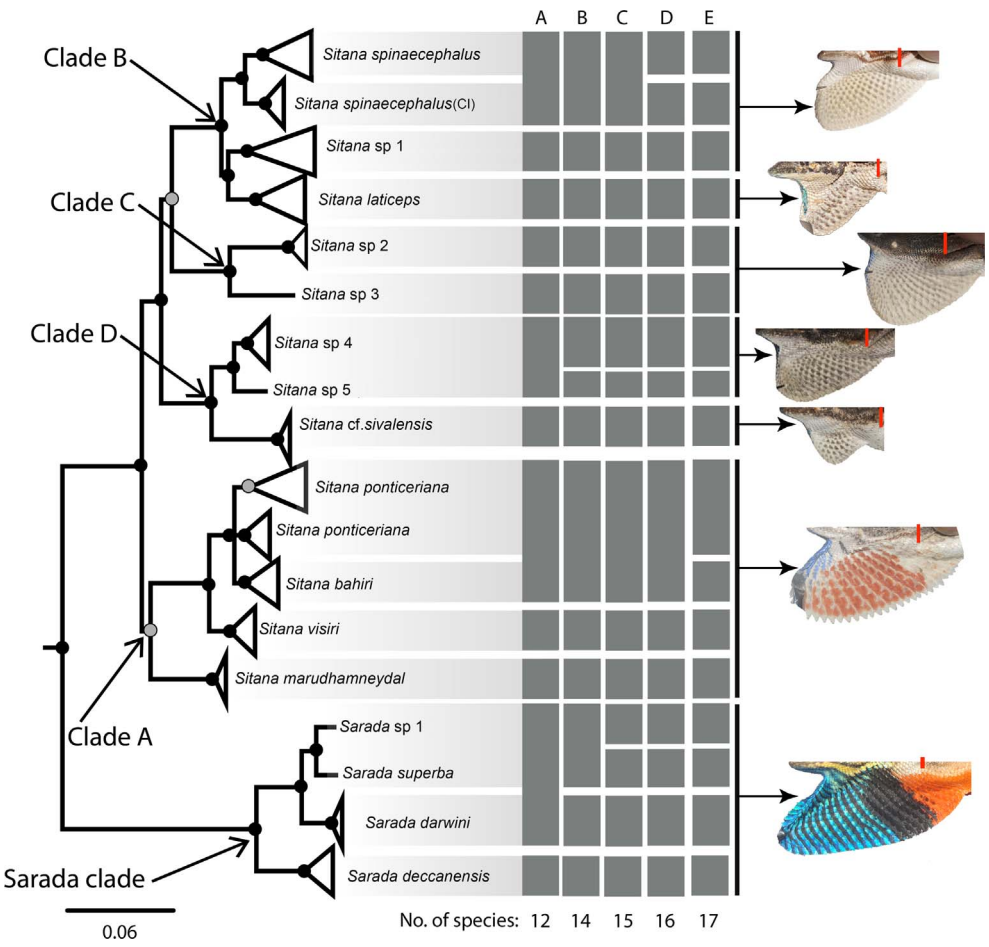

Fig. 1. Bayesian phylogram of FTL built using the concatenated dataset. Bayesian posterior probability is indicated at each node with dark circle representing > 0.95 support; gray circle < 0.95 support. A. GMYC species delimitation analysis, D. bPTP delimitation, B, C and E are models based on species distribution and current taxonomy. Representative dewlap type for species from each clade are shown. CI = Central Indian population.

59 mm and of the larger species (*Sarada superba*) was 68 mm (Fig. 3). The genus *Sitana* consisted of four well-supported clades viz; Clade A (*S. ponticeriana* clade), Clade B (*S. spinaecephalus* clade), Clade C (*Sitana* clade) and Clade D (*S. sivalensis* clade). Clade A contains species with medium to large body size (average SVL of smallest species (*S. ponticeriana*) and largest species (*S. marudhamneydhal*) were 42 mm and 50 mm respectively; Fig. 3) and breeding males have serrated dewlap with iridescent white, blue and orange spots (Fig. 1). Clade B includes species with small and medium bodied species (average SVL of smallest species (*S. laticeps*) and largest species (*S. spinaecephalus*) were 39 mm and 44 mm respectively; Fig. 3) dewlap with a single blue line (Fig. 1). Clade C includes two large bodied *Sitana* spp. (average SVL of smallest species (*S.* sp2) and largest species (*S.* sp3) were 49 mm and 50 mm respectively; Fig. 3) dewlap with a single blue line which is well spread in the lower jaw (Fig. 1). Clade D includes species of small and medium-sized *Sitana* spp (average SVL of smallest species (*S.* cf. *sivalensis*) and largest species *S.* (sp5) were 40 mm and 45 mm respectively; Fig. 3) they also have only a single blue line on the dewlap (Fig. 1).

3.2. Species delimitation

The single threshold GMYC method suggests that there are 12 species in the genera *Sarada* and *Sitana* (Fig. 2; Supplementary material box1). Whereas sixteen species were retrieved in the *Sarada – Sitana* complex by bPTP (Fig. 2). Most of the species delimitated by these methods received high posterior probability (Supplementary material, Fig. S3). In the *BEAST analysis, the marginal likelihood estimates were highest for the 15 species model compared to the other four models (Supplementary material, Fig. S4). Bayes Factor values suggest that the 15 species model is the best compared to all the other four models (Table 2). The 15 species model suggests that there are five new species of *Sitana* and one new species of *Sarada*. *Sitana bahiri* is nested within the widespread species *Sitana ponticeriana* and neither of the species delimitation methods (GMYC & bPTP) delimited *S. bahiri* as a distinct species. Furthermore, the species tree built in *BEAST considering *S. bahiri* as a separate species (17 species model) had the lowest MLE values (Supplementary material, Fig. S4). Morphological comparisons suggest that 13 out of the 15 species delimited here have diagnostic characters (Table 3). *Sitana laticeps* have overlap in morphological characters with *Sitana* sp1 and could potentially have distributional overlap in the southern range limits of *S. laticeps*. *Sitana bahiri* has overlapping morphological characters with *S. ponticeriana* (Table 3). *Sitana spinaecephalus* from central India, delimited as a separate species in the bPTP analysis, has extensive overlap in morphology and distribution with *Sitana spinaecephalus* (Table 3).

3.3. Divergence dating

Effective sample sizes (ESS) of all parameters were largely over 200 for the two independent BEAST runs, which indicated stationarity and adequate sampling sizes. The predominantly dry zone species of the *Sarada-Sitana* group diverged from the Sri Lankan wet zone *Otocryptis* (*O. wiegmanni*) around 26 mya (95% HPD: 19.5–31.4 mya) during the Oligocene and the separation of *Sitana* and *Sarada* occurred around 18 mya (95% HPD: 14.1–22.9) in early Miocene (Fig. 4a). The earliest diversification in *Sitana* started around 15–9 mya in the middle Miocene leading to the five major lineages (Fig. 4a). Subsequent lineage split happened around 10–3 mya in late Miocene and Pliocene leading to currently known and putative species (Fig. 4a). In the case of *Sarada,* the earliest divergence was around 6 mya (95% HPD: 4.2–9.6 mya) in

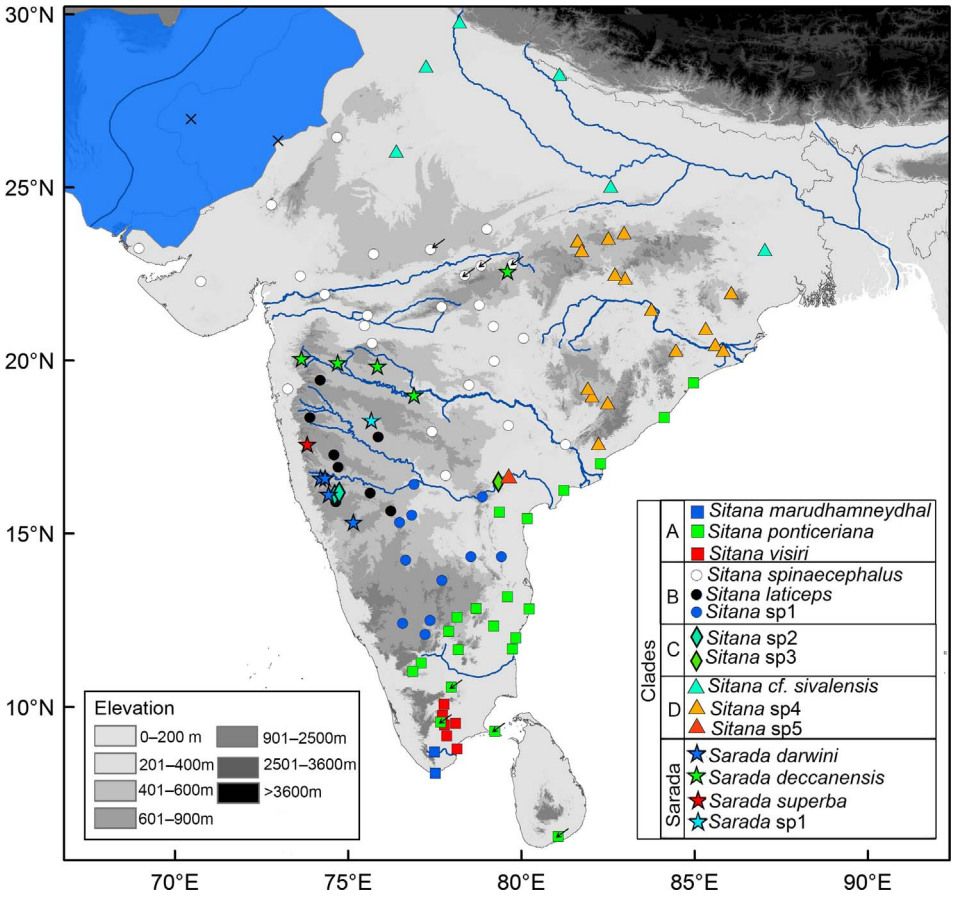

Fig. 2. Sampling localities for FTL species used in this study. Note: transparent blue shade represent regions with less than 350 mm rain in western India; cross marks are areas that were thoroughly sampled during FTL breeding season but were not seen; arrow indicates *Sitana bahiri* in the south and *Sitana spinaecephalus* in Central India, one or both of these populations were delimited as distinct species in the 16 & 17 species model (see Fig. 1). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

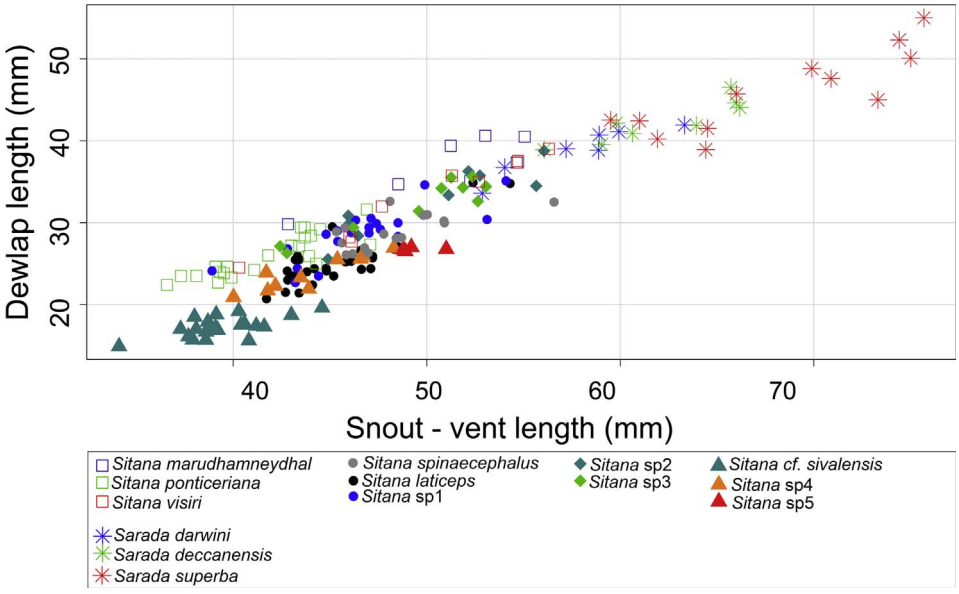

Fig. 3. Scatterplot showing dewlap size and body size of different FTL species. Note: we did not collect any male sample for *Sarada* sp1 during this study.

late Miocene leading to *S. decannensis*, the remaining three species diverged from their sisters more recently (3–2 mya) in Pliocene. Thus, much of the diversification within *Sitana* and *Sarada* occurred recently in the last 8–5 mya (Figs. 4a and b) during late Miocene and Pliocene. The gamma statistic value was positive (+0.04) suggesting an increasing trend in diversification towards the tip but it was not significant (two-tailed test P = 0.96).

4. Discussion

In the last two decades many new species have been described in the *Sitana* complex, this study has contributed six more putative species to this complex; five in *Sitana* and one in *Sarada*. Thus species diversity within fan-throated lizards has been vastly underestimated. Similar findings have been reported for other lizard groups (Agarwal and Karanth, 2015; Agarwal and Ramakrishnan, 2017) from the Indian dry zones. These studies indicate that the Indian dry zones have highly

Table 2
Pairwise model comparison using Bayes factor for the marginal Likelihood values obtained from path sampling (PS) and stepping stone (SS) sampling methods for the Beast analysis. Bayes factor greater than 3.2–10 is considered substantial and 10–100 is considered as strong support for one model over another.

	Path sampling					Stepping stone sampling				
Model (No. of species)	A (12)	B (14)	C (15)	D (16)	E (17)	A (12)	B (14)	C (15)	D (16)	E (17)
A (12)	0					0				
B (14)	6.63	0				5.7	0			
C (15)	12.76	6.14	0			12.56	6.87	0		
D (16)	5.8	−0.83	−6.97	0		4.86	−0.84	−7.7	0	
E (17)	−17.95	−24.58	−30.71	−23.74	0	−18.71	−24.4	−31.27	−23.57	0

underestimated diversity of lizards. The Indian dry zones constitute a vast expanse of open habitat which is climatically and geomorphologically heterogeneous thus providing an ideal setting for lineage diversification among these lizard groups.

4.1. Diversity and distribution

Most of the delimited species are geographically separated from their sister species but some have partial overlap (Fig. 2). None of the putative species were sighted from the same locality as their sister species. Most species except *Sarada superba, Sitana spinaecephalus* (in its southern limits) and *Sitana laticeps* (in its western limits) are distributed in areas which receive less than 1500 mm rainfall.

The four *Sarada* species are mostly found on the eastern side of Northern Western Ghats, these areas are dominated by lateritic plateaus and/or porous black soil (Figs. 2 and 5). Two out of the three species described in *Sarada* are known to use crevices in the black soil substrate to hide. Distribution of this genus appears to be limited by soil type in the south. Nine out of the eleven species of *Sitana* delimited in this study are distributed in Peninsular India (Fig. 1). *S. ponticeriana* clade (A) is distributed only in the east coast of India and coastal Sri Lanka. Out of the three species delimited in this clade (A), *Sitana ponticeriana* is found in India and Sri Lanka. The Moyar river gorge and the Nilgiri massif in the south-west, and the mouth of Mahanadi River in the north are barriers for distribution of this species (Figs. 2 and 5). *S. marudhamneydhal* is only found south of Tamirabarani River and *S. visiri* is found south of Cauvery River and has overlapping distribution with *S. ponticeriana*. *Sitana spinaecephalus* clade (B) has three species, *Sitana spinaecephalus* is the most widespread among the three, found in the plains of Peninsular India and parts of Northern India (Figs. 2 and 5). It is possible that the absence of this species beyond the Aravalli hills is because of the climatic barrier, as this region (Thar Desert) receives extremely low rainfall (Fig. 2). Furthermore, it is also possible that this niche was preoccupied with other diurnal fully/partially terrestrial lizards (*Trapelus, Ophisops* and *Acanthodactylus*) thus barring the expansion of this species. In northeast and east their range is bound by Maikal hills and the northern Eastern Ghats respectively which are forested (Figs. 2 and 5). *Sitana laticeps* has distribution in most of the Northern Deccan plateau and their distribution stops just south of Krishna river without any physical or climatic barrier. *Sitana* sp1 is another widespread species in this clade found just south of *S. laticeps* range (Fig. 2). The Moyar river gorge and the Nilgiri massif is a clear barrier for *Sitana* sp1 distribution in the south and in the east its distribution does not extend into the range of *S. ponticeriana* (Fig. 2).

Out of the three delimited species in clade D, *S.* cf. *sivalensis* is widespread and occupies most of the northern Indian plains (Fig. 2). This species is not found in the Aravalli hill range (Figs. 2 and 5) and east of Brahmaputra River (Figs. 2 and 5). *Sitana* sp 4 is distributed on the hills of Northeastern Peninsular India (Fig. 2). The Godavari basin could be a potential barrier for its distribution in the south. *Sitana* sp 5 was found only in two adjacent localities south of the Godavari and Krishna river basin (Fig. 2). Clade C consists of only two undescribed species (*Sitana* sp2 & sp3), both are point endemics in similar latitudes but 500 km apart. These two species are found on the rocky plateau south of Krishna river and its tributaries. Thus, a combination of landscape heterogeneity and climatic factors govern the current

Table 3
Pairwise comparison of the eight morphological characters and one geographic criterion (see species delimitation section in materials and methods) for different species pairs of *Sitana* and *Sarada*. Numbers correspond to one or more characters/criterion useful in separating species pairs. Note: dewlap characters were only used for male-male comparisons and counts comparisons are for either males or females.

S.no	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	*Sitana laticeps*	_																
2	*Sitana* sp1	0	_															
3	*Sitana spinaecephalus*	4,9	4,9	_														
4	*Sitana spinaecephalus* (Central India)	4,9	4,9	0	_													
5	*Sitana* sp2	1,9	1,9	4,9	4,9	_												
6	*Sitana* sp3	1,9	1,9	4,9	4,9	5,9	_											
7	*Sitana* sp4	9	5,9	2,4,9	2,4,9	2,5,9	2,5,9	_										
8	*Sitana* sp5	9	5,9	2,4,9	2,4,9	5,9	2,5	2,9	_									
9	*Sitana* cf. *sivalensis*	2,5,9	2,5,9	2,4,5,9	2,4,5,9	2,5,9	2,5,9	2,5,9	2,5,9	_								
10	*Sitana visiri*	1,3,4,9	1,3,4,9	1,3,4,9	1,3,4,9	1,3,4,9	1,3,4,9	1,3,4,9	1,3,4,9	1,3–5,9	_							
11	*Sitana marudhamneydhal*	1,3,4,9	1,3,4,9	1,3,4,9	1,3,4,9	1,3,4,9	1,3,4,9	1,3,4,9	1,3,4,9	1,3–5,9	2,5	_						
12	*Sitana ponticeriana*	1,3,4,9	1,3,4,9	1,3,4,9	1,3,4,9	1,3,4,9	1,3,4,9	1,3,4,9	1,3,4,9	1,3–5,9	2,5	2,5	_					
13	*Sitana bahiri*	1,3,4,9	1,3,4,9	1,3,4,9	1,3,4,9	1,3,4,9	1,3,4,9	1,3,4,9	1,3,4,9	1,3–5,9	2	5	0	_				
14	*Sarada deccanensis*	1,4–6,9	1,4–6,9	1,2,4–6,9	1,2,4–6,9	1,4–6,9	1,4–6,9	1,2,4–6,9	1,2,4–6,9	1,2,4–6,9	1,3–6,9	1,3–6,9	1–6,9	1–6,9	_			
15	*Sarada superba*	1,4–6,9	1,4–6,9	1,2,4–6,9	1,2,4–6,9	1,4–6,9	1,4–6,9	1,2,4–6,9	1,2,4–6,9	1,2,4–6,9	1,3–6,9	1,3–6,9	1–6,9	1–6,9	7,9	_		
16	*Sarada darwini*	1,4–6,9	1,4–6,9	1,2,4–6,9	1,2,4–6,9	1,4–6,9	1,4–6,9	1,2,4–6,9	1,2,4–6,9	1,2,4–6,9	1,3–6,9	1,3–6,9	1–6,9	1–6,9	7,9	8,9	_	
17	*Sarada* sp1	1,4–6,9	1,4–6,9	1,2,4–6,9	1,2,4–6,9	1,4–6,9	1,4–6,9	1,2,4–6,9	1,2,4–6,9	1,2,4–6,9	1,3–6,9	1,3–6,9	1–6,9	1–6,9	9?	9?	9?	_

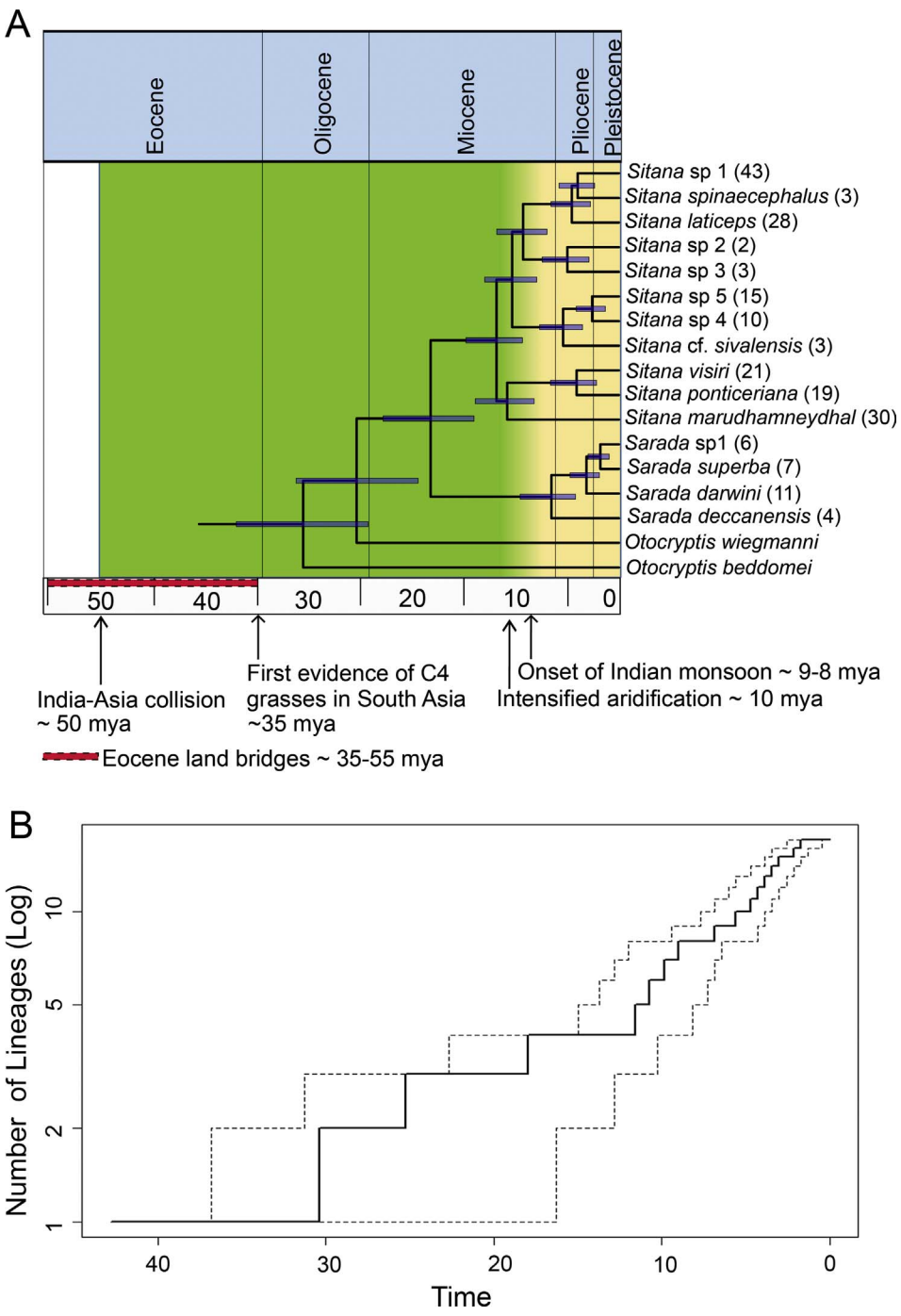

Fig. 4. (a) Beast chronogram of *Sitana* and *Sarada*. Node bars represent 95% HPD, numbers in parentheses are referenced in Fig. S1 and Table S1. Timings of the major geoclimatic events are pointed out with arrows at the bottom. Change in vegetation structure from forests (green) to an environment with a substantial amount of grass cover (yellow) in the Siwaliks, Pakistan (modified from Edwards et al. (2010)). (b) Lineages-through time (LTT) plot for the 15 species of *Sitana* & *Sarada* and the two *Otocryptis* species, 95% Confidence Intervals are shown. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

distribution of different *Sitana* and *Sarada* species.

4.2. Historical Biogeography

The genus *Otocryptis* from Sri Lanka was retrieved as sister to *Sarada-Sitana* which is consistent with previous reports (Macey et al. (2000), Fig. 4a). However, *Otocryptis* was paraphyletic with respect to *Sarada-Sitana*. In our time tree, the earliest divergence, around 31 mya, is between the lineages leading to Indian *Otocryptis beddomei* and *Otocryptis wiegmanni* from Sri Lanka + *Sarada-Sitana* (Fig. 4a), which is similar to the dates (~28 mya) of the split between *Otocryptis wiegmanni* and *Sitana* reported in Grismer et al. (2016). This was followed by the divergence between *Otocryptis wiegmanni* and *Sarada-Sitana* around 18 mya. Thus, the ages of the basal nodes in the two *Otocryptis* lineages + *Sarada-Sitana* clade falls well within the Oligocene and early Miocene when much of India and Sri Lanka was covered with wet forests. *Otocryptis* has two deeply divergent lineages and both are wet forest species whereas the more recently evolved lineages of *Sarada-Sitana* are predominantly dry zone species.

Our molecular dating analysis suggests that *Sarada* and *Sitana* clade started diversifying during Mid-Miocene. Several agamids in West Asia, Central Asia, Australia and Africa also underwent diversification during the Miocene (Hugall and Lee, 2004; Guo and Wang, 2007; Shoo et al., 2008; Leaché et al., 2014; Tamar et al., 2016). These diversification events correspond to shifts in climatic conditions globally during the Miocene (Zachos et al., 2001). However, much of the diversification in the *Sarada* and *Sitana* clades occurred from late Miocene onwards with a steep increase in lineage diversification in the last 8–5 my (Fig. 4a).

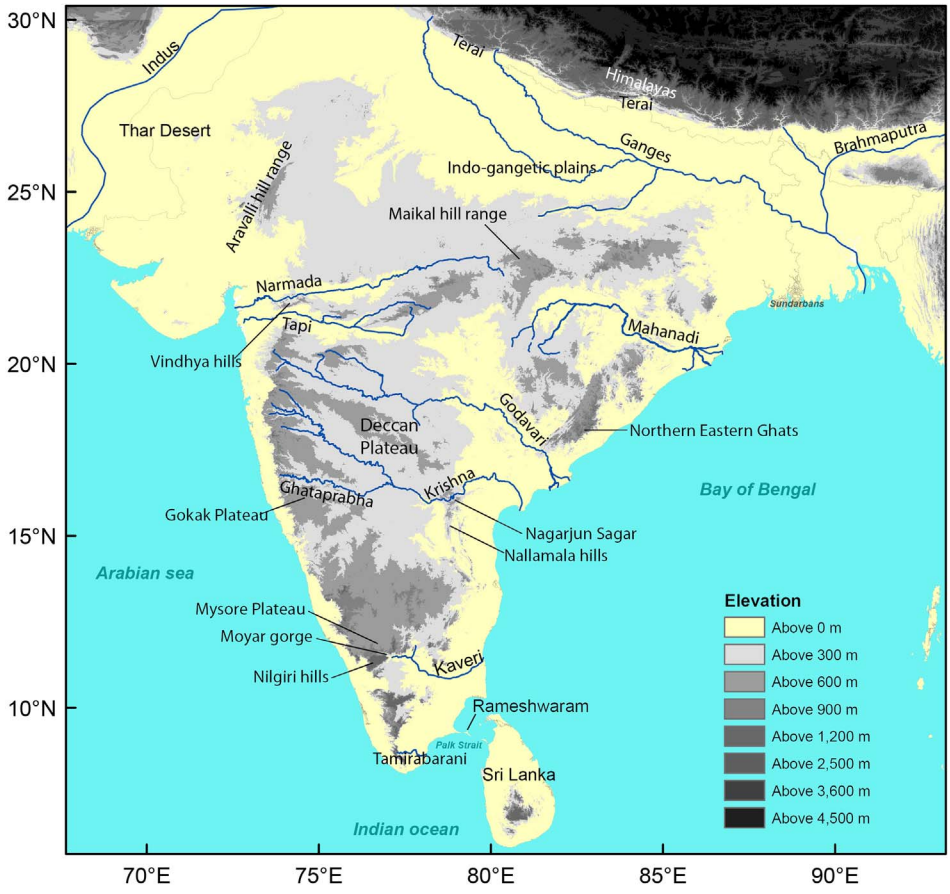

Fig. 5. Map showing physiographic features of the Indian subcontinent in the distributional range of FTL. Major rivers and potential barriers for different clades and for some species referred in the text are labeled.

The LTT plot also hints at this pattern, wherein lineages accumulation is very low till about 20 mya followed by an increase in diversification in more recent times (10 my onwards, Fig. 4b). Interestingly, this period coincides with the time when open habitats were expanding in South Asia and climatic conditions were transforming from cool moist to hot arid environments (Edwards et al., 2010). This was also the period of intensification of monsoon climate characterized by seasonal weather conditions. These climate change events caused the retreat of humid forest to relict patches along Southwest India and Sri Lanka and a concomitant spread of open habitats (Karanth, 2003). Thus, with the advent of monsoon-driven climate change and the associated aridification, the rainforest adapted lineage *Otocryptis* may have experienced extinction events or lack of speciation whereas it's dry-adapted sister lineages of *Sitana* and *Sarada* underwent diversification. The extant *Otocryptis* are most likely relict representatives that are restricted to isolated patches of predominantly lowland rainforest habitats of the Western Ghats and Sri Lanka. Additionally, *Otocryptis* consists of only three species and therefore appear depauperated compared to 18 species in *Sarada-Sitana*.

The dewlap color in the *Otocryptis-Sarada-Sitana* complex also provides an indirect evidence for the humid forest origin of this group. It has been hypothesized that agamid species with colorful dewlap tend to occur in forested areas while those with little color live in open habitats (Ord et al., 2015). The forest-dwelling *Otocryptis* possess colorful dewlap whereas most *Sitana* have very little coloration. Nevertheless, all *Sarada* are brightly colored (an outlier for the hypothesis). Ord et al (2015) argue that although present-day *Sitana* occupies open habitats, they almost certainly originated from a forest-dwelling ancestor. Taken together these observations suggest that the change from forests to open habitats has influenced fan-throated lizards' diversification.

Two other recent studies have also invoked Miocene climate change as an important driver that has shaped the current diversity of lizard fauna in this region (Agarwal and Karanth, 2015; Agarwal and Ramakrishnan, 2017). The notable contrast between fan-throated lizards and another terrestrial lizard group, lacertids (*Ophisops*), is that the latter are already arid zone adapted species which dispersed from the Saharo-Arabian region and radiated in India (Agarwal and Ramakrishnan, 2017). *Sitana* and *Sarada* on the other hand, are evidently derived from a lineage which was wet zone adapted and the ancestral stock dispersed from the east (Grismer et al., 2016) which later adapted to the dry conditions. Our species delimitation models and external morphology do not support *S. bahiri* as a distinct species from *Sitana ponticeriana*. The present-day populations of *Sitana ponticeriana* are found in Rameshwaram and intermittent islets between India and Sri Lanka, suggesting that during periods of low sea level this land bridge (Voris, 2000) would have facilitated movement of fauna between India and Sri Lanka.

5. Conclusions

In conclusion, our study shows a vast underestimated diversity of fan-throated lizards in India which were delineated using species delimitation models. This study establishes a link between late Miocene aridification and diversification of a terrestrial lizard in the Indian subcontinent. As a result of fine-scale sampling in this study, we identify potential geographic and climatic barriers for fan-throated lizards which can be verified for other small terrestrial animals. It is also evident that Peninsular India has a higher diversity of fan-throated lizards (13 species) compared to rest of the subcontinent probably due to the heterogeneity in the landscape which allowed isolation of populations. *Sitana* cf. *sivalensis* is found throughout the Indo-Gangetic plains, the other two species described from this clade *S. schleichi* and *S. fusca*, have subtle morphological difference and need further verification. Key diagnostic characters used in differentiating species in *Sarada* and

Sitana are dewlap type and size (Deepak et al., 2016a). Previous studies suggest that the underlying driver of dewlap variation in FTLs is sexual selection (Kamath, 2016). Thus, isolation of population due to habitat heterogeneity and sexual selection might have influenced speciation in this group. This study highlights the evolutionary significance of the arid zone, a long-ignored landscape in India, which falls outside the flagship diversity hotspots such as the Western Ghats.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ympev.2017.11.016.

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